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Effect of Ractopamine HCl Supplementation on Fecal Shedding of *Escherichia coli* O157:H7 and *Salmonella* in Feedlot Cattle

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Abstract. The effects of the β-agonist ractopamine, recently approved for use in feedlot cattle to improve carcass quality and performance, on fecal shedding *Escherichia coli* O157:H7 and *Salmonella* in feedlot cattle was examined. In the first study, 20 feedlot steers and heifers were randomly assigned to receive ractopamine or no ractopamine (control) by way of oral bolus for 28 days. Fecal samples were collected daily, and shedding of *E. coli* O157:H7 determined. When examined during the entire 28-day experimental period, ractopamine decreased ($P = 0.0006$) the percentage of cattle shedding *E. coli* O157:H7 (58% vs. 42% for control and ractopamine treatments, respectively). A second study was conducted in a commercial feedlot facility in the southwestern United States. Eighteen pens of cross-bred beef heifers (approximately 100 head/pen and 9 pens/treatment) were randomly assigned to receive either 0 (control) or 200 mg ractopamine/head·d^{-1}. Fresh fecal samples (30/pen) were collected off the pen floor before ractopamine supplementation and again after approximately 28 days of ractopamine supplementation (within a few days of slaughter); the samples were cultured for *E. coli* O157:H7 and *Salmonella*. The percentage of animals shedding *E. coli* O157:H7 was decreased when data were pooled across replicates ($P = 0.05$) in ractopamine-treated cattle compared with controls. The percentage of animals shedding *Salmonella* tended to be higher ($P = 0.08$) with the ractopamine treatment when data were pooled across replicates. Although further research is required to confirm these results, the potential food safety implications of this research are intriguing.

Quorum sensing is a mechanism of cell-to-cell signaling involving hormone-like compounds, called autoinducers, used by the bacteria to sense their own populations as well as the populations of other bacteria [1]. This communication system has been called ''a global regulatory mechanism for basic physiologic functions of *Escherichia coli* O157:H7'' and is involved in gene expression, pathogenesis, metabolism, growth and division, and protein biosynthesis [1] and has been speculated as being the ''language'' that host cells and bacteria use to communicate [2]. The catecholamine hormones have been reported to be involved in a bacterial quorum-sensing system that regulates virulence gene expression of *E. coli* O157:H7 [2] and have been reported to stimulate the growth of intestinal *E. coli* [3] and *E. coli* O157:H7 [4–6].

Ractopamine hydrochloride is a synthetic β-agonist that repartitions nutrients to increase the ratio of lean-to-adipose tissue [7, 8] and improves performance, carcass leanness, and dressing percentage in finishing pigs [9, 10]. Ractopamine was recently approved by the United States Food and Drug Administration for use in finishing

Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the United States Drug Administration and does not imply its approval to the exclusion of other products that may be suitable.

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beef cattle, to be fed the last 28 days of the finishing period for similar performance and carcass improvements. Interestingly, the physiologic counterparts to synthetic β-agonists are the hormones norepinephrine and epinephrine [11]. We conducted a study in which yearling beef cattle, naturally infected with *E. coli* O157:H7, received ractopamine for a period of 28 days based on our hypothesis that feeding ractopamine may either directly, or by way of increased plasma norepinephrine, increase gut populations and fecal shedding of *E. coli* O157:H7. Based on the unexpected findings of the first experiment reported herein, a second experiment was conducted examining the effects of ractopamine supplementation on the fecal prevalence of *E. coli* O157:H7 and *Salmonella* in beef cattle in a commercial feedlot.

Materials and Methods

Experiment I. Twenty yearling cross-bred beef steers and heifers (with sex equally represented in treatments) were used in this experiment. All animals were grouped in a single, outdoor pen with access to shade and were gradually adapted to an 80:20 concentrate-and-hay diet during a period of 2 weeks. Animals were group fed at approximately 3% body weight (BW) each morning and had a mineral supplement, salt, and water available for *ad libitum* consumption. Cattle were randomly assigned to one of two treatments (10 head/treatment): control (empty gelatin capsule) or ractopamine (20 mg / head) administered daily for 28 days *per os*.

Animals were run through a squeeze chute daily for administration of treatments and sample collection. Fecal samples were collected daily by way of rectal palpation for bacterial isolation described later. Blood samples were collected by way of jugular venipuncture on days 0, 14, and 28 for determination of plasma catecholamine and serum melatonin concentrations. Blood samples for plasma catecholamines were kept on ice until centrifugation (3,000 × g for 15 minutes.), and the plasma was decanted and frozen (–80°C). Concentrations of epinephrine and norepinephrine were determined by competitive enzyme immunoassay (EIA) using duplicate 10-µl aliquots of ethylenediaminetetraacetic acid–plasma (Bi-CAT EIA; American Laboratory Products, Windham, NH). Epinephrine and norepinephrine were extracted from plasma by use of a cis-diolspecific affinity gel. The extracts were acylated to N-acylepinephrine and N-acylnorepinephrine; these were then converted enzymatically to N-acylmetanephrine and N-acylnometanephrine, respectively, during the detection step of the EIA. The reaction was monitored by use of a microplate reader at a wavelength setting 450 nm. The standard curve for epinephrine was 0, 1, 4, 16, 64, and 256 ng/ml epinephrine. The norepinephrine standard curve was comprised of 0, 4, 16, 64, 256, and 1024 ng/ml norepinephrine. Plasma concentrations of catecholamines were determined by use of the absorbance data for the standard curves and the AssayZap software package for competitive binding assays (BioSoft).

Blood samples collected for melatonin assay were allowed to clot at room temperature; were centrifuged as described previously; and the serum was removed and frozen. Serum melatonin was determined using a double antibody radioimmunoassay (RIA). The RIA used components of a commercial kit (melatonin direct RIA; Buhlmann Laboratories AG, Schonenbuch, Switzerland), and the procedure was performed as described by the manufacturer. Melatonin was determined in two assays having an average within-assay CV of 11.6% and a between-assay CV of 5.8%. BWs were recorded on days 0, 7, 14, and 21 (the scale was not operational on day 28).

Feedlot experiment. The effect of ractopamine supplementation on feedlot cattle in a commercial feedlot in the southwestern United States was examined during the summer of 2005 (June through August). Eighteen pens of cross-bred beef heifers (approximately 100 head/pen and 9 pens/treatment) were randomly assigned to receive either 0 (control) or 200 mg ractopamine/hd·d^{-1}. Ractopamine was incorporated in the total-mixed ration per the manufacturer's recommendations and fed to the cattle for 28 days immediately before slaughter. Treatment starting dates were staggered (approximately 2 weeks) such that there were three replicates (six pens/replicate) in the experiment. Fresh fecal samples (30/pen) were collected off the pen floor using sterile palpation sleeves before ractopamine supplementation and again within a few days of slaughter (approximately 28 days after initiation of ractopamine supplementation). Care was taken to collect fecal samples from different animals that were not contaminated with feedlot soil. Fecal samples were placed on ice and shipped overnight to our laboratory in College Station, TX, for processing the next day.

Bacterial culture, isolation, and enumeration methods. Bacterial culture of all samples collected in Experiment I was initiated within 2 hours of sample collection. *E. coli* O157:H7 culture and isolation was conducted on all samples using immunomagnetic separation (IMS) technique. Ten grams feces was enriched in 90 ml Gram-negative broth containing vancomycin (8 µg/ml), cefixime (0.5 µg/ml), and cefsoludin (10 µg/ml) and incubated (for 6 hours at 37°C). After incubation, 20 µl anti-*E. coli* O157:H7 antibody-labeled paramagnetic beads (Neogen, Lansing, MI) were added to 1-ml volumes of the enrichments, mixed, and washed according to IMS separation techniques described previously [12]. Fifty microliters of the resulting suspension was spread-plated on CHROMagar O157 (DRG International, Mountain Side, NJ) plates (containing 2.5 µg/ml potassium tellurite) and incubated overnight (37°C). Pink colonies exhibiting typical *E. coli* O157:H7 morphology were resuspended in phosphate-buffered saline (PBS; pH 6.5) and confirmed as *E. coli* O157:H7 using the Reveal microbial screening test according to the manufacturer's instructions (Neogen Corp., Lansing, MI).

Generic *E. coli* was quantified by serially diluting 1 g feces in sterile PBS followed by plating on CHROMagar *E. coli* agar. Plates were incubated (24 hours at 37°C), and blue colonies were manually counted. *Salmonella* (feedlot experiment only) was cultured by enriching approximately 10 g feces in 90 ml tetrathionate broth (37°C for 24 hours), followed by a second enrichment in Rapport-Vassilidis broth (100 µl in 5 ml at 42°C for 24 hours). Enrichments were plated on brilliant green agar (Oxoid, Hampshire, UK) supplemented with novobiocin (25 µg/ml) and incubated (37°C for 24 hours). After incubation, colonies exhibiting typical *Salmonella* morphology were confirmed biochemically using lysine and triple sugar iron agars. Positive samples were restreaked on tryptic soy agar with 5% sheep blood (Becton, Dickenson, Sparks, MD) for further confirmation, and serogrouping was conducted using slide agglutination with *Salmonella* O antiserum, poly A-I and Vi, and groups C1 and E1 (Difco, Detroit, MI). *Salmonella* isolates were stored (–80°C) using CryoCare bacterial preservers (Key Scientific Products, Round Rock, TX).

Statistical analysis. Data were analyzed using SAS version 8.02 (SAS, Cary, NC). The incidence of fecal shedding of *E. coli* O157:H7 was subjected to chi-square analysis using the PROC FREQ procedure. For experiment I, fecal *E. coli* O157:H7 shedding data were grouped and analyzed by week of the experiment and across weeks. Data for

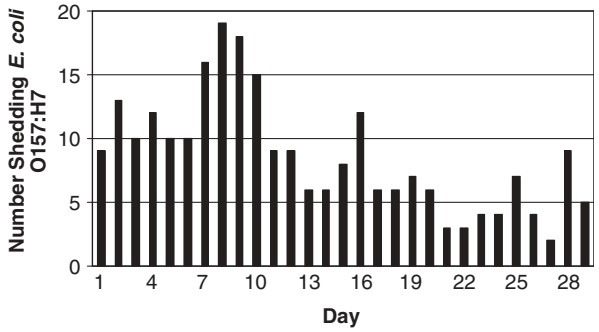

Fig. 1. Number of animals shedding *E. coli* O157:H7 across treatments during the 28-day experimental period. Sampling = 20 animals/d (experiment I).

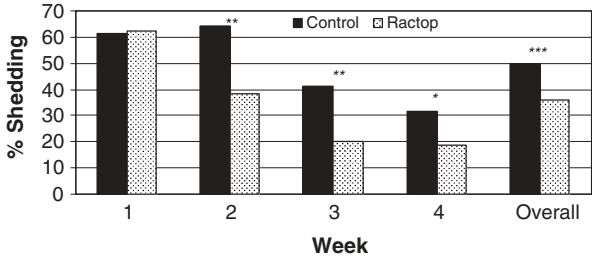

Fig. 2. Effect of ractopamine supplementation (Ractop) on the percentage of animals shedding *E. coli* O157:H7 by week and during the 28-day experimental period. Sampling = 20 animals/d and 140 samples weekly/treatment (experiment I). *$P < 0.10$. **$P < 0.01$. ***$P < 0.001$.

fecal shedding of generic *E. coli*, catecholamine, and melatonin concentrations were analyzed using the PROC mixed procedure with treatment, day, and the treatment-x-day interaction included in the model. BW data were subjected to analysis of variance appropriate for a completely randomized design. Differences among means were considered significant at a 5% level of significance.

Results

Experiment I. Intermittent shedding patterns of fecal *E. coli* O157:H7 were observed throughout the experimental period regardless of treatment, with a gradual decrease in the number of positive shedders occurring as the study progressed (Fig. 1). Overall, the percentage of cattle shedding *E. coli* O157:H7 during the 28-day experimental period was lower ($P = 0.0006$) in the ractopamine-treated cattle (41.8%) compared with control animals (58.2%). No differences ($P = 0.87$) were observed during the first week of the study; however, during the second ($P = 0.002$) and third ($P = 0.006$) weeks, the percentage of cattle shedding *E. coli* O157:H7 was lower in cattle receiving ractopamine (Fig. 2). This same tendency ($P = 0.08$) was observed during the fourth week of the study, and although the same percentage of shedders versus nonshedders was observed as in week 2, the difference was not significant because of the decrease in the overall number of animals shedding during this time period (Fig. 2).

Ractopamine-treatment tended ($P = 0.09$) to increase fecal populations of generic *E. coli* (6.25 vs 5.97 CFU g/feces $\log_{10}$) compared with control animals when examined across sampling days. No treatment-x-day interaction ($P = 0.91$) was observed. Generic *E. coli* counts were within the same $\log_{10}$ at each sampling time with the exception of day 28, when counts were one log higher in ractopamine-treated cattle (data not shown).

Plasma catecholamine and serum melatonin concentrations are presented in Fig. 3. No treatment or treatment-x-day effects ($P > 0.10$) were observed for plasma epinephrine, norepinephrine, or serum melatonin, although a day effect ($P < 0.01$) was observed for plasma epinephrine and serum melatonin concentrations. Norepinephrine concentrations were numerically

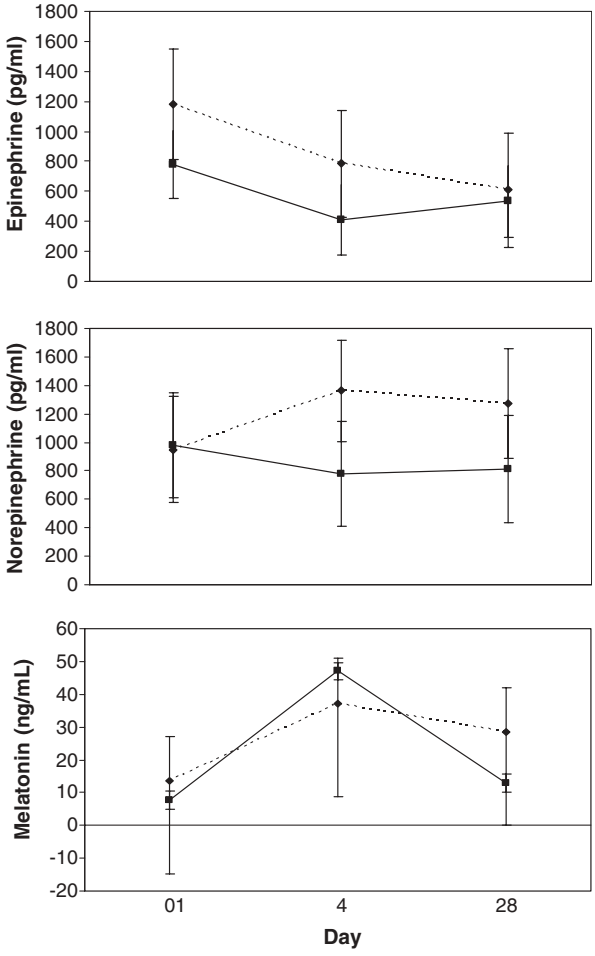

Fig. 3. Plasma epinephrine, norepinephrine, and serum melatonin concentrations on days 0, 14, and 28 in control cattle (solid line) and cattle dosed with ractopamine (dashed line) for 28 days (experiment I).

higher on both days 14 and 28; however, the large degree of variation prevented detection of statistically significant differences.

BWs were similar ($P = 0.83$) at the initiation (298 vs. 294 kg for control and ractopamine treatments, respectively) of the experiment. Although ractopamine-treated animals gained more weight during the study period, the differences were not significantly different (data not shown).

Feedlot experiment. Fecal shedding data for *E. coli* O157:H7 and *Salmonella* are presented in Table 1. Overall, across treatments and collection times, the incidence of *E. coli* O157:H7 and *Salmonella* was 21.0% and 21.7%, respectively. Throughout the experiment there was a high degree of variation in the percentage of positive samples at the different collection times, ranging from 0% to 82.9% for *E. coli* O157:H7 and 0% to 60.2% for *Salmonella*. Before ractopamine supplementation, significant differences were noted among pens within treatment. In the first replicate, a higher percentage of fecal samples from pens assigned to the ractopamine treatment were positive for *E. coli* O157:H7 ($P = 0.02$) and *Salmonella* ($P = 0.004$) than control pen samples. In the second replicate, a greater ($P = 0.0005$) percentage of samples from control pens were positive for *Salmonella*. After ractopamine supplementation, no differences ($P > 0.10$) in the percentage of samples positive for *E. coli* O157:H7 were noted for the first two replicates; however, a significant decrease in *E. coli* O157:H7 was observed in the third replicate ($P = 0.02$), and when prevalence data were combined for all replicates ($P = 0.05$). Likewise, no differences in the percentage of samples positive for *Salmonella* were noted in replicates one or two after ractopamine supplementation. However, contrary to the *E. coli* data, a significant increase in the percentage of *Salmonella*-positive samples was observed during the third replicate within the ractopamine treatment, and when replicate data were combined, ractopamine treatment tended ($P = 0.08$) to increase the percentage of positive *Salmonella* samples compared with the control treatment.

Discussion

To our knowledge, this is the first research examining the effect of ractopamine supplementation on foodborne pathogens in feedlot cattle. Previous *in vitro* research reported the "stimulatory" effects of the catecholamines on *E. coli* O157:H7 [2, 5, 6] and led us to hypothesize that a similar compound, ractopamine, may act likewise, increasing gut concentrations and fecal shedding of *E. coli* O157:H7. The first experiment was conducted at our laboratory with beef cattle individually dosed with ractopamine daily for 28 days and "naturally" shedding *E. coli* O157:H7 in the feces. After 1 week of initiating ractopamine treatments, we saw a decrease in the number of animals shedding *E. coli* O157:H7, and this decrease continued throughout the 4-week experimental period. Because of a decrease in the overall numbers of animals shedding *E. coli* O157:H7 in the last week of the experiment, this decrease tended to be significant and followed the same trend observed in weeks 2 and 3.

We examined plasma catecholamines in an attempt to elucidate possible explanations for the observed shedding differences. Plasma norepineprine concentrations were numerically higher on days 14 and 28 in the ractopamine treatment; however, variation among the animals, although stress was kept to a minimum, and the animals were conditioned to handling before blood collection on day 14, was high. Others have likewise reported no differences in catecholamine concentrations after administration of a β-agonist to cattle [13]. Previous work in our laboratory demonstrated an effect of exogenous melatonin on fecal shedding of *E. coli* O157:H7 in beef cattle [14]. Norepinephrine is reported to regulate pineal α_1-adrenoreceptors [15], which increases melatonin secretion [16, 17]. If ractopamine produced a similar increase in melatonin secretion, it might explain the decrease in fecal shedding of *E. coli* O157:H7 observed in the present study, similar to that observed previously in melatonin-treated cattle. However, no treatment differences were observed in serum melatonin in the present study.

In planning further research with ractopamine, we discovered a typographic error in the information sheet used to calculate our ractopamine dose used in experiment I. Unfortunately, the dose used in experiment I was much lower than the recommended dose for feedlot cattle. Nevertheless, the unexpected results were encouraging and led to the feedlot experiment to examine the effects of ractopamine supplementation on *E. coli* O157:H7 and *Salmonella* on a larger scale and in a commercial feedlot setting. We examined >2,100 fecal samples during the course of the feedlot study for the pathogens *E. coli* O157:H7 and *Salmonella*. Although prevalence rates were high at some of the collection times, overall the prevalence of *E. coli* O157:H7 (21%) in this experiment was similar to levels reported by others [18, 19]. *Salmonella* prevalence (21.7%) reported in this study was higher than reported by others in feedlot cattle [20, 21]. Although every effort was made to collect fresh fecal pats free of environmental con-

Table 1. Percentage of feedlot fecal samples positive for *E. coli* O157:H7 and *Salmonella* before and after approximately 28 days of ractopamine supplementation[a]

Collection	Replicate	*E. coli* O157;H7			*Salmonella*		
		Control	Ractopamine	*P>F*	Control	Ractopamine	*P>F*
Initial	1	67.4	82.9	0.02	38.9	60.2	0.004
	2	18.9	15.6	0.55	20	3.3	0.0005
	3	2.2	0	0.16	0	0	
	Average	29.4	32.5	0.44	19.6	20.9	0.71
Final	1	10.0	11.1	0.81	7.8	4.5	0.36
	2	13.5	7.8	0.22	12.4	13.3	0.85
	3	17.8	6.7	0.02	40	61.1	0.005
	Average	13.8	8.5	0.05	20.1	26.4	0.08

[a]Each percentage represents 90 samples collected from 3 pens (30/pen) of approximately 100 crossbred beef heifers/pen.

tamination, representing 30 different animals/pen, we cannot rule out the possibility that multiple samples from individual animals may have been collected. The prevalence of both pathogens appeared to be directly impacted by feedlot conditions, with levels much higher when muddy conditions were encountered during sample collection.

Ractopamine supplementation decreased the incidence of *E. coli* O157:H7 in the third replicate and overall when data from all three replicates were pooled. Although significant differences were not observed in the first two replicates, the decrease in the percentage of *E. coli* O157:H7–positive samples was greater for cattle in the ractopamine treatment. This observed decrease, although exciting from a food safety standpoint, is difficult to explain microbiologically. Based on previous research with catecholamines, we expected to see an increase in shedding, if any effect at all. It is possible that slight changes in gastrointestinal motility, function, or microbial ecology caused by ractopamine treatment were significant enough to influence *E. coli* O157:H7 populations. Norepinephrine has been reported to augment adherence to intestinal epithelia in the ligated bovine ileal loop model of infection [22], and perhaps ractopamine acted in a similar fashion, increasing adherence of *E. coli* O157:H7 to the gut epithelium and thereby decreasing fecal shedding.

The effects of ractopamine supplementation on *Salmonella* shedding observed in this study were also previously unknown. Ractopamine produced an effect on *Salmonella* opposite to that observed for *E. coli* O157:H7, increasing *Salmonella* in the third replicate and tending to increase the overall prevalence compared with controls. Reasons for this increase are unclear but may be related to the decrease in *E. coli* O157:H7 and possibly other Gram-negative bacteria, which may have provided *Salmonella* a competitive advantage in the gastrointestinal tract. However, in the first experiment, generic *E. coli* tended to increase in ractopamine-treated cattle, indicating that other factors are more likely involved.

Herein, we provide the first report examining the effects of ractopamine supplementation on fecal shedding of two important foodborne pathogens in beef cattle, *E. coli* O157:H7 and *Salmonella*. Because ractopamine is designed to be fed immediately before slaughter, the potential food safety implications of this research are substantial; however, continued research is needed to further confirm these findings. Decreasing the amount of pathogenic bacteria entering the abbatoir could have a significant impact on potential carcass contamination and cases of human foodborne illness.

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